11:30 a.m.

821-3 **Amlodipine Decreases Myocardial Oxygen Consumption via Angiotensin 4 Receptor: A Unique Effect of the R+ Enantiomer**

Seema Mital, Edward Shesely, Thomas H. Hintze, *Columbia University, New York, New York, New York Medical College, Valhalla, New York.*

Background: Amlodipine decreases myocardial oxygen consumption (MVO2) via R+ enantiomer mediated endothelial nitric oxide (NO) release. We investigated the role of angiotensin (AT) 2 and 4 receptors in mediating this effect.
Methods: We measured MVO2 in vitro using the Clark type oxygen electrode in isolated LV myocardial segments obtained from (i) wild-type and AT2 receptor knock-out (KO) mice (n=5), and (ii) explanted failing human hearts obtained at the time of heart transplantation (n=2). We studied the effect of increasing doses of R+ enantiomer (10-7M -10-5 M) on MVO2 with and without (i) NO synthase inhibitor, nitro-L-arginine methyl ester (L-NAME, 10-3 M), (ii) AT2 receptor blocker, PD 123319, 10-6-10-5M, and (iii) specific AT4 receptor blocker, divalyl angiotensin 4,10-5M.
Results: Wild-type mice: R+ caused a dose-dependent decrease in MVO2 (-24±8% at highest dose, p<0.05). This effect was inhibited by L-NAME (-17±7%) and 10-5M PD 123319 (2±7%). AT2 KO mice: R+ caused a dose-dependent decrease in MVO2 (-25±3% at highest dose, p<0.05) and this was not blocked by 10-6M PD123319, selective AT2 receptor antagonist suggesting an alternate receptor/pathway. At higher concentration of 10-5M, PD123319 blocked the effect of R+ (-5±2%, p<0.01). At this dose, PD compound appears to be a nonselective AT receptor antagonist possibly acting on AT4 receptor. L-NAME caused a 33% reduction in the effect of R+. Human myocardium: R+ decreased MVO2 with a -20±1% decrease at highest dose. This effect was attenuated by both L-NAME and 10-5M PD123319. Also, specific AT4 receptor blocker caused a 50% attenuation of R+ effect. Conclusion: The R+ enantiomer of amlodipine decreases MVO2 by AT4 receptor mediated NO release in AT2 receptor knock-out mice. In failing human myocardium, this effect also appears to be mediated by AT4 receptors.

11:45 a.m.

821-4 **Angiotensin II Receptor Antagonism and ACE Inhibition Ameliorate Hyperinsulinemia and Obesity in a Murine Model of Polygenic Obesity**

Jan R. Ortlepp, Jutta Breuer, Karin Kluge, Reinhard Kluge, Hans-Georg Joost, *Medical Clinic I RWTH University Hospital, Aachen, Germany, Institute for Pharmacology and Toxicology, Aachen, Germany.*

Background: ACE inhibitors are well established in the prevention of hypertension-associated complications of the metabolic syndrome. This study was performed in order to assess the effects of the ACE inhibitor captopril and of the angiotensin II receptor antagonist irbesartan on other conditions of the metabolic syndrome in an animal model.
Methods: Male NZO/BL6 F1 mice were treated with captopril, irbesartan, or placebo for ten months. Treatment with captopril and irbesartan in equivalent dosage was controlled by monitoring the blood pressure (BP). At the end of the study, gain of body weight (BW), serum levels of insulin, cholesterol, triglycerides and creatinine, cardiac weight and degree of atherosclerosis were determined.
Results: Control animals treated with placebo developed a metabolic syndrome with obesity (55.5 ± 6.3 g), hypertension (146 ± 10 mmHg), hyperinsulinemia (7.2 ± 5.7 ng/ml), hypercholesterolemia (5.1 ± 0.7 mmol/l), cardiac hypertrophy (269 ± 44 mg) and atherosclerotic plaques in the ascending aorta (3.6 ± 1.5 µm²). Treatment with ACE inhibitor or angiotensin II receptor antagonist significantly (p<0.001) reduces hypertension (73 ± 5 and 78 ± 11 mmHg), cardiac hypertrophy (203 ± 26 and 202 ± 18 mg) and atherosclerosis (2.2 ± 0.9 and 1.8 ± 0.8 µm²). In addition captopril and irbesartan prevented the development of obesity (42.2 ± 3.5 and 38.3 ± 2.8 g) and hyperinsulinemia (3.6 ± 1.5 and 1.8 ± 0.4 ng/ml), Irbesartan being somewhat more effective than captopril in the prevention of hyperinsulinemia (p<0.01).
Conclusion: In a mouse model of the obesity associated metabolic syndrome, long term treatment with an ACE inhibitor or an angiotensin II receptor antagonist, can ameliorate obesity and hyperinsulinemia.

Noon

821-5 **Chronic Angiotensin II (AT1) Receptor Antagonism Selectively Enhances Renal cGMP Production With Improved Renal Function in Experimental Overt Congestive Heart Failure**

Horng H. Chen, John B. Osborn, Gail J. Harty, John C. Burnett, Jr., *Mayo Clinic and Foundation, Rochester, Minnesota.*

BACKGROUND: Recent studies have reported that preservation of renal function is an important predictor of survival in congestive heart failure (CHF). A hallmark of overt CHF is an attenuated renal cGMP production to endogenous natriuretic peptides (NPs) and nitric oxide (NO), which may lead to decline in renal function. Studies have also shown that Angiotensin II (ANG II) activates cGMP phosphodiesterase resulting in increased cGMP degradation. We therefore hypothesized that chronic AT1 receptor antagonism would restore the renal cGMP production in response to endogenous NPs and NO as assessed by urinary cGMP with improved sodium excretion.
METHODS: We determined the cardiorenal actions of chronic AT1 blockade (Valsartan, Novartis, 320 mg daily for 10 days, n=5) in a canine model rapid ventricular pacing induced overt CHF (245 bpm for 10 days) as compared to a non-treated group (n=5).
RESULTS: After 10 days of chronic AT1 receptor antagonism, urinary sodium excretion increased (12.4 ± 3.3 vs 2.7 ± 1.3 uEq/min,p<0.05) in association with a marked increase in urinary cGMP excretion (1558 ± 200 vs 139 ±65 pmol/min, p<0.05) as compared to the non-treated group. The natriuretic response to chronic AT1 receptor antagonism was localized to the inner medullary collecting duct by the lithium clearance technique, a nephron site rich in NPs receptors and sensitive to NO, as distal tubular fractional sodium reabsorption decreased in the AT1 blocker group vs non-treated group (97.6 ± 0.3 vs 98.9 ± 0.5 %, p<0.05). These renal responses where selective as they occurred in the absence of any changes in plasma NPs or cGMP. Chronic AT1 receptor antagonism also reduced cardiac filling pressures consistent with cardiac unloading.
CONCLUSION: We conclude that chronic AT1 receptor antagonism in experimental overt CHF enhances renal cGMP production, the common secondary messenger for the NPs and NO system resulting in improved renal tubular function and sodium excretion. This study provides insight into renal and humoral pathophysiological actions of ANG II and the AT1 receptor in CHF and mechanisms by which AT1 receptor antagonism may mediate beneficial therapeutic properties by targeting the kidney in this disease state.

POSTER SESSION

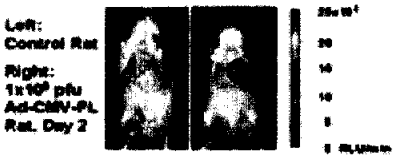
1105 Cardiovascular Gene Expression, Delivery, and Inhibition

Monday, March 18, 2002, Noon-2:00 p.m.
Georgia World Congress Center, Hall G
Presentation Hour: Noon-1:00 p.m.

1105-71 **Optical Imaging of Adenoviral Mediated Cardiac Gene Expression in Living Rats**

Joseph C. Wu, Masa Inubushi, Heinrich Schelbert, Sanjiv S. Gambhir, *UCLA School of Medicine, Los Angeles, California.*

Background: Direct injection of adenovirus into the heart is useful for studying reporter gene constructs. However, most studies rely on postmortem analysis. We have validated a novel method of studying rat cardiac gene expression of CMV driven firefly luciferase (Ad-CMV-FL) utilizing a cooled Charged Couple Detector (CCD) camera.
Methods: Rats underwent a standard thoracotomy. In one group, $1x10^9$ pfu was injected into left ventricular wall (n=3). Another group received serially diluted titers ($1x10^9$ to $1x10^5$ pfu). Control rats were injected with $1x10^9$ pfu of Ad-CMV-HSV1-sr39tk expressing mutant thymidine kinase (n=3). Images were acquired on days 2 and 5 after i.p. injection of luciferin (125 mg/kg) and data expressed as relative light unit per minute (RLU/min).
Results: Rats imaged serially show cardiac FL activity of 172,423 ± 8,066 (day 2) and 252,755 ± 83,739 RLU/min (day 5). Rats injected with diluted titers show considerable FL activity at day 5: 1,452 RLU/min ($1x10^7$ pfu) and 248 RLU/min ($1x10^6$ pfu). All values are statistically significant (p<0.05) compared to control rats showing background signals (10±5 RLU/min).
Conclusion: In summary, this study demonstrates the feasibility of imaging the location, magnitude, and persistence of cardiac reporter gene expression in rats over time. The cooled CCD camera produces consistent results and the detection sensitivity is very high, down to $1x10^6$ pfu. This is the first demonstration of imaging cardiac gene expression in a living subject.



1105-72 **DNA Chip Analysis of Akt-Regulated Genes in Endothelial Cells Reveals Activation of Many Proangiogenic Pathways**

Hyo-Soo Kim, Yuri Ivashchenko, Kenneth Walsh, *Boston University Medical Center, Boston, Massachusetts, Seoul National University Hospital, Seoul, South Korea.*

Background: The serine-threonine protein kinase Akt1 is activated downstream of numerous angiogenic factors in endothelial cells. To understand the role of this signaling pathway in the angiogenic response, we used adenovirus-mediated Akt1 gene transfer and high-throughput affymatrix oligonucleotide microarrays to examine the Akt-regulated genes in human umbilical vein endothelial cells (HUVEC).
Methods: HUVEC were either mock-infected or infected with adenoviral vectors expressing constitutively-active Akt1 or β-galactosidase (moi=100). Under these conditions, transfection efficiency was 95% or greater. At 24 hours post-treatment, RNAs were isolated, labelled during reverse-transcription, and hybridized to oligonucleotide microarrays, the Human Genome U95A array. The genes that showed a significant change of expression (>2-fold increase or decrease) in both replicate and duplicate assays were selected and confirmed by quantitative PCR.
Results: Constitutive activation of Akt signaling altered the expression of 130 genes of a total of 12,000 genes analyzed. Consistent with the pro-angiogenic role attributed to Akt, there were many angiogenesis-related growth factors and cytokines that were induced by an increase in Akt signaling. These include VEGF-A, VEGF-C, IL-8, GRO(Growth Regulated Oncogene)-α, GRO-β, GRO-γ, Cox2, HOX, and heme oxygenase-1. Akt signaling also induced the expression of adhesion molecules (VCAM-1, ICAM-1, ELAM-1) associated with endothelial cell activation. As expected from the documented pro-survival action of Akt, several anti-apoptotic genes (HSP70) were increased. Akt also increased a series of genes involved in the cholesterol synthesis. Finally, Akt exagger-